

## Commentary

# Australasian Guideline (2nd Edition): an Annex to the CLSI and UK Guidelines for the Performance of the Sweat Test for the Diagnosis of Cystic Fibrosis

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### Summary of Recommendations

This document is a comment on the most recent Clinical Laboratory Standards Institute (CLSI) and United Kingdom (UK) multidisciplinary guidelines for the conduct of sweat testing to aid in the diagnosis of cystic fibrosis (CF). This commentary emphasises important details that must be followed in order to support best practice and obtain accurate results. The document expands on details not clearly described elsewhere, providing the following recommendations for sweat chloride and conductivity analysis.

- Recommendation 1. Following newborn screening (NBS), babies with two cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations detected have CF but should also have a sweat chloride test to confirm the diagnosis given the life-long implications of a diagnosis of CF. Babies with one *CFTR* mutation identified require a sweat chloride test to distinguish healthy carriers from affected babies with an unidentified second mutation.
- Recommendation 2. Patients presenting with clinical signs of CF should have a sweat test; see 1998 US CF Foundation consensus document on the diagnosis of CF for a list of clinical manifestations.
- Recommendation 3. The laboratory should endeavour to perform and report a sweat test within five working days of receipt of the request following positive NBS.
- Recommendation 4. Measurement of the sweat chloride concentration is the key investigation for the diagnosis of CF and should be the only analyte considered for diagnostic purposes.
- Recommendation 5. Measurement of sweat sodium, osmolality and conductivity are not acceptable tests for the diagnosis of CF.
- Recommendation 6. Sweat conductivity, usually measured by the Wescor SWEAT CHEK™ following Macroduct® sweat collection, may be used as a screening test only. It is not appropriate to do conductivity post a positive NBS.
- Recommendation 7. Sweat tests should not be performed until the subject is greater than two weeks corrected age and weighs more than 2 kg.
- Recommendation 8. Sweat testing should be delayed if the patient is acutely unwell, dehydrated, oedematous, malnourished or does not have a suitable skin site free of eczema.
- Recommendation 9. It is a sound practice to prepare a sweat test information sheet for patients and families.
- Recommendation 10. Sweat stimulation and collection is covered by consistent statements in the two guidelines (UK 2014 and CLSI 2009) which should be consulted for explicit details. These statements equally apply to sweat chloride and conductivity stimulation and collection.
- Recommendation 11. In addition to chloridimetry, inductively coupled plasma mass spectrometry (ICP-MS) and ion chromatography/high performance liquid chromatography (IC-HPLC) are appropriate for sweat chloride analysis and are also potential reference measurement procedures.

- Recommendation 12. Use of the Nanoduct® is not recommended until more research, peer comparison and product development information is available.
- Recommendation 13. For infants up to 6 months of age, we advocate the following reference interval (decision limit): sweat chloride >60 mmol/L supports the diagnosis of CF; a patient is unlikely to have CF when the sweat chloride is ≤29 mmol/L; and patients with an intermediate sweat chloride result of 30–60 mmol/L may be CF and should be referred to a physician experienced in the diagnosis of CF for clinical evaluation.
- Recommendation 14. For children from 6 months-of-age through to adults, we advocate a reference interval (decision limit): sweat chloride >60 mmol/L supports the diagnosis of CF; a patient is unlikely to have CF when the sweat chloride is ≤39 mmol/L; and patients with an intermediate sweat chloride result of 40–60 mmol/L may be CF and should be referred to a physician experienced in the diagnosis of CF for clinical evaluation:
- Recommendation 15. All patients with an elevated sweat conductivity (>50 mmol/L) should be referred for a sweat chloride test.
- Recommendation 16. Sweat conductivity should be reported clearly and the report should state the value of measured conductivity in standard ‘sodium chloride equivalent’ units, mmol/L.
- Recommendation 17. Each person trained to carry out sweat testing should perform at least 10 sweat tests annually, and detailed training records must be fully documented.
- Recommendation 18. Training records should be kept up to date in accordance with local audit agency requirements.
- Recommendation 19. Local accreditation authorities should ensure assessors with expertise in sweat testing are included in the assessor panel.
- Recommendation 20. Local collection of sweat with transport to another laboratory for analysis requires experimental demonstration that the samples remain stable during transport.

## Introduction

Cystic fibrosis (CF) is an autosomal recessive disease resulting from mutations in the cystic fibrosis transmembrane

conductance regulator (*CFTR*) gene.<sup>1</sup> Absent or reduced *CFTR* function results in altered electrolyte transport at epithelial surfaces, in particular the airways, pancreatic and biliary ducts, the gastrointestinal tract, vas deferens and the eccrine sweat ducts. The main clinical features are progressive suppurative lung disease and pancreatic exocrine insufficiency. Other clinical features include bowel obstruction, biliary cirrhosis, male infertility (absent vas deferens) and dehydration.<sup>2</sup> The elevated sweat chloride concentration forms the basis of the sweat test that has been used for diagnostic purposes since 1959.<sup>3</sup> More recently, *CFTR* mutation analysis has provided another method of diagnostic testing.<sup>4</sup> Over 2000 *CFTR* sequence variations have been identified, although the majority are rare.<sup>5</sup> Many clinical laboratories can routinely test for 20–50 mutations, accounting for more than 85% of mutations in the Australasian population.<sup>6</sup> Despite advances in genetic testing, the sweat test remains important for the diagnosis of CF.<sup>7</sup>

CF had an incidence in Australia and New Zealand (NZ) of approximately 1:2500 live births but as preconception screening and other pregnancy screening options become more common, most screening laboratories are seeing a lower incidence, as low as 1:3500.<sup>8–10</sup> The majority (90–95%) of new patients are detected by newborn screening (NBS) programmes.<sup>8,9</sup> In Australia and NZ NBS involves measurement of immunoreactive trypsinogen (IRT) on day 2–4 of life, followed by limited *CFTR* mutation analysis for babies with IRT above the 99<sup>th</sup> percentile.<sup>9</sup> Babies with two mutations detected have CF but should also have a sweat test to confirm the diagnosis given the life-long implications of a diagnosis of CF. Babies with one *CFTR* mutation identified require a sweat test to distinguish healthy carriers from affected babies with an unidentified second mutation. Any person presenting with clinical features of CF, including meconium ileus, or a family history of CF (first degree relative) should also have a sweat test regardless of age or newborn screening result.<sup>11,12</sup>

Both false-positive and false-negative sweat tests can have detrimental life-long consequences. Sweat test methods must be standardised to ensure consistent quality for diagnostic accuracy.<sup>13</sup>

The last Australia and NZ guideline for the performance of the sweat test was published in 2006.<sup>14</sup> This drew heavily on the existing guidelines published by the CLSI in the US and the multidisciplinary sweat test working party in the UK. The CLSI guideline was updated in 2010 (approved guideline C34-A3) and the UK guideline in 2014.<sup>15,16</sup> This working party has employed the UK guideline as a major resource because of its detail and evidence-based format.

The aim of this guideline is to provide recommendations to support standardisation and best practice for (a) sweat conductivity screening; and (b) sweat chloride testing to diagnose and monitor CF. This document is a comment on the most recent CLSI and UK guidelines, and the recommendations for the performance of the sweat test supersede the Australasian guideline of 2006. These comments are applicable to all laboratories performing sweat tests in Australasia, in particular those enrolled in the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP). In this document we emphasise important details that must be followed in order to support best practice and obtain accurate results, and to expand on details not clearly described elsewhere. A detailed account of how to perform a sweat test can be found in the CLSI (2009) and UK (2014) guidelines which should be consulted if further details are required.<sup>15,16</sup> It is available on-line ([www.acb.org.uk](http://www.acb.org.uk)). A comparison of the new Australian and NZ (2017), CLSI (2009) and UK (2014) guidelines, along with the latest Cystic Fibrosis Foundation consensus document,<sup>17</sup> is provided in the Table.

### Indications for Sweat Testing

- a. The majority of patients for testing are identified by a positive NBS test for CF (that is, an elevated IRT, followed by the detection of one or two *CFTR* mutations). NBS screening *CFTR* mutation panels vary in each state of Australia and NZ with the aim of detecting approximately 90% of affected babies. The screening panels are usually more limited than the expanded diagnostic panels. *CFTR* sequencing is not included as part of NBS in Australia and NZ. The *CFTR* mutations sought in screening are those most common in a European population, so babies of other ethnicities with a different mutation spectrum may not be identified. Although CF is believed to be rare in Aboriginal, Torres Strait Island, Polynesian and Asian populations, more requests for sweat tests from persons of these ethnicities are being received by laboratories in Australia and NZ.

The CLSI and UK guidelines do not make a recommendation about how promptly a sweat test should be performed following a positive newborn screening result. Parents are anxious once they have been notified of the positive NBS test and the fact that the majority will turn out to be carriers is of no immediate comfort. While the system of referral is not under the control of the laboratory, once a request is received, performance of the test and conveying the result to parents is a matter of urgency. It is recommended that the test be performed and the authorised result made available to the requestor within five working days where practical.

- b. Patients presenting with ***clinical signs of CF*** (see 1998 US CF Foundation consensus document on the diagnosis of CF for a list of clinical manifestations) should have a sweat test.<sup>11,12,17</sup> Such patients may arise from cases missed by the screening programme because the IRT was below the cut-off value or because they carried mutations not included in the NBS panel. Other possibilities are migrants from countries without a screening programme or those born before screening was commenced (NBS for CF started in 1981 in New South Wales and NZ, with Western Australia the last Australian state to commence in 2001). In Australia, 98% of children have NBS, some missing out because of early hospital discharge or parental refusal of screening.<sup>18</sup> There is a broad phenotype that may be consistent with *CFTR* disease (e.g. absent vas deferens, sinusitis or recurrent pancreatitis) so that laboratories are also seeing an increasing number of adults referred for sweat testing.<sup>19</sup>
- c. ***Family history of CF***. Siblings of patients with CF have a 1 in 4 chance of being affected. They may have been missed by NBS or not presented with clinical features that have prompted a sweat test previously. As such, all siblings of patients with CF should have a sweat test.
- d. ***Monitoring of therapy with ivacaftor*** is required under the Australian Pharmaceutical Benefits Scheme. This is likely to become a more frequent cause of referrals when other *CFTR* potentiators and correctors become licenced for use.<sup>20,21</sup>

**Recommendation 1.** Following newborn screening, babies with two *CFTR* mutations detected have CF but should also have a sweat chloride test to confirm the diagnosis given the life-long implications. Babies with one *CFTR* mutation identified, require a sweat chloride test to distinguish healthy carriers from affected babies with an unidentified second mutation.

**Recommendation 2.** Patients presenting with clinical signs of CF should have a sweat test; see 1998 US CF Foundation consensus document on the diagnosis of CF for a list of clinical manifestations.

**Recommendation 3.** The laboratory should endeavour to perform and report a sweat test within five working days of receipt of the request following positive NBS.

### Acceptable and Non-acceptable Tests

Measurement of the sweat chloride concentration is the key investigation for the diagnosis of CF and should be the only

analyte considered for diagnostic purposes. Some laboratories have continued to measure sweat sodium as well, often as a check of the validity of the chloride value, or alternatively to provide the clinician with a sodium/chloride ratio. There are, however, no established criteria as to what constitutes acceptable values, so this practice is no longer recommended.<sup>22</sup> In accordance with this, the RCPAQAP provides EQA only for sweat chloride and conductivity concentration.

Sweat conductivity is related to the concentration of all ions in sweat and is higher than sweat chloride by a mean of about 15 mmol/L.<sup>23</sup> Conductivity can be measured in an acceptable manner with the Wescor system.<sup>23,24</sup> It is the recommendation of the Sweat Test Advisory Committee that sweat conductivity be used as a screening test and not a diagnostic test. This is consistent with the CLSI and UK guidelines. All patients with an elevated sweat conductivity should be referred for a sweat chloride test. In addition, all infants less than 6 months of age should be referred for a sweat chloride test irrespective of the conductivity result.

Sweat conductivity, usually measured by the Wescor SWEAT CHEK™ following Macroduct® sweat collection, may be used as a screening test only. We do not recommend the Wescor Nanoduct® because it was not approved for use in the UK guideline nor discussed by CLSI. Measurement of sweat osmolality is not an acceptable test for the diagnosis of CF.

**Recommendation 4.** Measurement of the sweat chloride concentration is the key investigation for the diagnosis of CF and should be the only analyte considered for diagnostic purposes.

**Recommendation 5.** Measurement of sweat sodium, osmolality and conductivity are not acceptable tests for the diagnosis of CF.

**Recommendation 6.** Sweat conductivity, usually measured by the Wescor SWEAT CHEK™ following Macroduct® sweat collection, may be used as a screening test only. It is not appropriate to do conductivity post a positive NBS.

### Who is Suitable to Test?

Sweat tests should not be performed until the subject is greater than two weeks corrected age and weighs more than 2 kg. This is because there are often technical problems in doing the test in very small infants and there may be a greater risk of complications (see below) or obtaining insufficient sweat.<sup>25</sup> Practically, in Australia and NZ, most babies with positive NBS results are identified around 3–4 weeks of age.<sup>8,9</sup> Occasionally, the test may be attempted in younger smaller babies, provided there are good clinical reasons for doing so.<sup>26</sup>

Sweat testing is contra-indicated in babies less than 48 hours of age because high concentrations of sweat electrolytes can be found on the first day of life and sweat volume is low.<sup>27</sup>

Sweat testing should be delayed if the patient is acutely unwell, dehydrated, oedematous or malnourished. It may not be possible to cease fludrocortisone or topirimate before doing a sweat test, but clinicians should be aware that these drugs can affect the sweat electrolyte values. There is no literature on the effect of systemic corticosteroids. Skin sites with eczema or serous exudation should be avoided as they impair sweat duct function or cause falsely high results by contamination with serum.

**Recommendation 7.** Sweat tests should not be performed until the subject is greater than two weeks corrected age and weighs more than 2 kg.

**Recommendation 8.** Sweat testing should be delayed if the patient is acutely unwell, dehydrated, oedematous, malnourished or does not have a suitable skin site free of eczema.

### Patient Preparation and Possible Complications

The purpose of the sweat test and how it will be carried out must be explained carefully to the parents of the child or the adolescent/adult being tested. They should be made aware that there is a small risk of complications. The most common observation is of mild reddening of the skin, which is of no consequence. However damage to the skin (such as burns and blistering) occurs very infrequently and is usually due to poor contact of the stimulation electrode with the skin causing an increase in electrical resistance and therefore heat build-up. This risk can be minimised by careful attention to technique (see next section) and to careful maintenance of electrode surfaces. It is a sound practise to prepare a sweat test information sheet for parents (see example sheet at <https://www.schn.health.nsw.gov.au/parents-and-carers/fact-sheets/sweat-test> and Appendix).

**Recommendation 9.** It is a sound practise to prepare a sweat test information sheet for patients and families.

### Sweat Stimulation and Collection

This is covered by consistent statements in the two guidelines which should be consulted for explicit details. These statements apply equally to sweat chloride and conductivity stimulation and collection. The matters considered critical by the Sweat Test Advisory Committee are the following:

- Sweat must be collected after stimulation by pilocarpine iontophoresis using either the Gibson and Cooke method or the Wescor Macroduct® system.



- The power supply must be a battery and should include a safety cut-out. All equipment must be checked on a 12-monthly basis and maintained in good working order. The current should be increased gradually to maximum of 2.5–4 mA and monitored throughout the procedure, which needs to be maintained for 3–5 minutes to stimulate the recommended quantity of sweat.
- Electrodes are usually made from stainless steel, copper or carbon and must be of a proper size to fit the patient's limb. They must be regularly cleaned and inspected and kept free of surface oxidation. When using the Wescor Macroduct® system, the manufacturer's instructions should be followed.
- The flexor surface of the forearm is the preferred site for sweat collection. Other sites such as the upper arm or thigh may be used if the arms are too small. The site must be free of skin disorders such as eczema.
- The electrolyte solutions to be used include US Pharmacopoeia grade pilocarpine nitrate (0.2–0.5%). This may be used at both electrodes. Alternatively, a solution of magnesium sulphate (0.05%) or potassium sulphate (1%) can be used at the cathode. Solutions containing chloride should be avoided because of the risk of contamination. Pilocarpine may be used in the form of a gel, as with the Wescor Macroduct® system.
- The following may be used for sweat collection:
  - (i) Gauze pads which have been repeatedly washed in distilled water to remove any traces of chloride.
  - (ii) Filter paper e.g. Whatman no. 42/44.  
Once applied to the stimulated area of skin for sweat collection, the gauze pads or filter paper should be covered with a sheet of impervious material that is sealed on all sides with tape to prevent evaporation.
  - (iii) Macroduct® disposable collector.
- Pads placed under the electrodes and soaked with the electrolyte solutions must be thick (3–8 thicknesses of hospital lint) and maintained in full contact with the skin. They must be about 1 cm larger than the electrodes in all dimensions to avoid electrode contact with the skin and the risk of burns.
- Sweat should be collected for not less than 20 min and no more than 30 min, unless the Macroduct® tubing is full. Great care should be taken to prevent contamination and evaporation.
- The minimum sweat secretion rate should not be less than 1 mg/m<sup>2</sup>/min over the collection period. The area function refers to the area of the stimulation electrode and collection device (Gibson and Cooke method), so this area must be known. *Insufficient volumes of sweat should not be pooled* but rather the test should be repeated. If this is necessary, a different site on the limb should be chosen. Sweat chloride analysis should not be performed if <0.075 g of sweat is obtained using the Gibson Cooke method, or <15 µL using the Macroduct® system over 30 min. Quantities lower than these should be reported as 'insufficient collection, please repeat test' or similar. The weight or volume of sweat collected from each patient must be recorded and reported. The sweat test laboratory should aim to keep inadequate collections below 5% of all collections, bilateral or otherwise. If insufficient samples are more than 5%, reviews of electrolyte concentrations, stimulation and collection area, instrument function, staff training, and patient hydration are warranted.
- If insufficient sweat is collected, only one repeat stimulation (on the same day) may be performed, using an alternative site, usually the opposite arm. Do not re-stimulate the same site. If the sweat volume is still insufficient, the test should be re-scheduled for another date.
- Some laboratories collect sweat from both arms simultaneously in order to minimise the number of 'quantity not sufficient' collections. This also allows the comparison of sweat chloride from one arm to be compared with the other as a way of documenting test quality. The 95<sup>th</sup> percentile difference is about 15 mmol/L so a difference greater than this value should be regarded with caution.<sup>28</sup> These collections should not be pooled.

**Recommendation 10.** Sweat stimulation and collection is covered by consistent statements in the two guidelines (UK 2014 and CLSI 2009) which should be consulted for explicit details. These statements equally apply to sweat chloride and conductivity stimulation and collection.

### Methods of Analysis

The UK guideline supports the use of colourimetry, coulometry (chloridometer) and ion selective electrode (ISE) for chloride analysis, as long as whatever method is used is fully validated. ISE analysis requires the addition of a known volume of saline to bring the chloride concentration to a range measurable by ISE, and this is a potential source of error. The CLSI guideline discusses only analysis by chloridometer.

There are now several laboratories measuring chloride by inductively coupled plasma mass spectrometry (ICP-MS), and this has been shown to be highly accurate.<sup>29</sup> Another recently published accurate method is ion chromatography/high performance liquid chromatography (IC-HPLC).<sup>30</sup> Both are appropriate for sweat chloride analysis and are also potential reference measurement procedures.

Wescor Inc has introduced a much smaller device, the Wescor Nanoduct®. This stimulates sweat glands by pilocarpine iontophoresis and sweat is collected and analysed *in situ* with a disposable sensor. This sensor is of small size and low sample requirement (3–5 µL), making it attractive for testing neonates.<sup>31,32</sup> Losty *et al.* (2006) found a high number of false-negatives (25%), thought to be due to a faulty batch of sensors.<sup>33</sup> The Nanoduct® was not discussed in the CLSI guideline and is not recommended in the UK guideline. Therefore, the Sweat Test Advisory Committee does not recommend the use of the Nanoduct® until more research, peer comparison and product development information is available.

**Recommendation 11.** In addition to chloridimetry, ICP-MS and IC-HPLC are appropriate for sweat chloride analysis and are also potential reference measurement procedures.

**Recommendation 12.** Use of the Nanoduct® is not recommended until more research, peer comparison and product development information is available.

### Decision Limits

There is accumulating evidence that values for sweat chloride are more age-related than previously identified, and the recommendations of both guidelines reflect this.<sup>34,35</sup> In addition, it is clear that there is a spectrum of CFTR diseases, from classic cystic fibrosis with pancreatic exocrine insufficiency to those with CF but who are pancreatic sufficient and those with isolated organ involvement, such as pancreatitis and absence of the vas deferens. There is some relationship with genotype class and often sweat chloride in each of these CFTR disease categories.<sup>36</sup> Patients with isolated organ involvement are not considered to have CF, and the term CFTR-related disease is used. There have been various generations of diagnostic guidelines from the Australian Paediatric Respiratory Group, US CF Foundation and the European CF Society.<sup>11,17,37-39</sup> In each of these, the complexity of the diagnosis is increasingly apparent. The foundations of the diagnosis, however, remain an assessment of the clinical features, NBS results, genotype and sweat chloride value. As such, creating absolute sweat chloride cut-offs for diagnosis is somewhat artificial. It is best to consider sweat chloride as a decision limit that determines the need for a more thorough clinical assessment. The most

recent US CF Foundation guideline on the diagnosis of CF (2017) have done away with age-related sweat chloride values and taken <30 mmol/L as the normal range with >60 mmol/L being consistent with a diagnosis of CF in all age groups.<sup>39</sup> It is the opinion of the Sweat Test Advisory Committee that this is not consistent with our clinical experience and the published data.<sup>34,35</sup> It is rare for patients with pancreatic-insufficient or -sufficient CF to have sweat chloride <40 mmol/L after infancy.<sup>36</sup> As such, we still advocate a decision limit for infants (up to 6 months), most likely to be tested after identification by NBS and a decision limit for children from 6 months of age through to adulthood. This serves the vast majority of patients having sweat testing.

### Decision Limits for Sweat Chloride Infants <6 months

Sweat chloride >60 mmol/L supports the diagnosis of CF.

Sweat chloride 30–60 mmol/L may be CF (referral to physician experienced in the diagnosis of CF is recommended for clinical evaluation, repeat sweat chloride testing and genotype analysis is recommended).

Sweat chloride ≤29 mmol/L unlikely to have CF.

### Decision Limits for Sweat Chloride Infants ≥6 months, Children, Adults

Sweat chloride >60 mmol/L supports the diagnosis of CF.

Sweat chloride 40–60 mmol/L may be CF (referral to physician experienced in the diagnosis of CF is recommended for clinical evaluation, repeat sweat chloride testing and genotype analysis is recommended).

Sweat chloride ≤39 mmol/L unlikely to be CF.

**Recommendation 13.** For infants up to 6 months of age, we advocate the following reference interval (decision limit): sweat chloride >60 mmol/L supports the diagnosis of CF; a patient is unlikely to have CF when the sweat chloride is ≤29 mmol/L; and patients with an intermediate sweat chloride result of 30–60 mmol/L may be CF and should be referred to a physician experienced in the diagnosis of CF for clinical evaluation.

**Recommendation 14.** For children from 6 months of age through to adults, we advocate a reference interval (decision limit): sweat chloride >60 mmol/L supports the diagnosis of CF; a patient is unlikely to have CF when the sweat chloride is ≤39 mmol/L; and patients with an intermediate sweat chloride result of 40–60 mmol/L may be CF and should be referred to a physician experienced in the diagnosis of CF for clinical evaluation.

### Decision Limits for Conductivity

At conductivity values >50 mmol/L, CF cannot be excluded. It is therefore appropriate for laboratories using conductivity to have a single decision limit of 50 mmol/L above which

patients should be referred for sweat chloride (or the laboratory reflex to this if available). Below 50 mmol/L, no further action is required on the basis of the sweat test if the patient is >6 months of age. These results should be reflected in interpretative comments.

The choice of decision limits and interpretive comments are the subject of regular audit by the Sweat Test Advisory Committee.

**Recommendation 15.** All patients with an elevated sweat conductivity (>50 mmol/L) should be referred for a sweat chloride test.

### Interpretative Remarks

Interpretive remarks incorporated into the report must be overseen by a senior scientist experienced in sweat testing, in consultation with an appropriate pathologist or clinician. The UK guideline provides an excellent list of comments which may be employed by laboratories on their reports to assist clinicians in result interpretation.

### Reporting Units

Sweat chloride should be reported in mmol/L.

In a recent survey by the Sweat Test Advisory Group it was apparent that some laboratories report conductivity in units of the expected chloride concentration, calculated from the sample conductivity.<sup>40</sup> This calculation is presumably based on the formula suggested by Hammond.<sup>23</sup> This practice causes uncertainty to the referrer about what has been tested, leading to undesirable variation in diagnostic strategy. The Sweat Test Advisory Committee recommends that conductivity be reported clearly and that the report states the value of measured conductivity in standard 'NaCl equivalent' units (i.e. the molar concentration of a pure sodium chloride solution having the same conductivity); using mmol/L.

Conductivity is expressed as mmol/L throughout this paper. This is an abbreviation of mmol/L (equivalent NaCl), a unit that represents the molar concentration of a solution of sodium chloride that has the same conductivity as the sweat sample at the same temperature. It is emphasised that this value does not in any way represent the actual sodium or chloride concentration in the sweat sample.

**Recommendation 16.** Sweat conductivity should be reported clearly and the report should state the value of measured conductivity in standard 'sodium chloride equivalent' units, mmol/L.

### Sample Quality Across the Total Testing Process

There is evidence that poor performance of sweat chloride

testing can lead to misdiagnosis.<sup>13,41-43</sup> The step of sweat testing most susceptible to incorrect performance is operator competency. Internal quality control (IQC) and EQA programmes are valuable for the control of analysis, analytical competency and interpretation of results, but cannot test the ability of the operator to collect the sweat accurately which is where a considerable amount of variation occurs. One way of testing all components of the sweat test is to perform a sweat test on selected staff volunteers at regular intervals. This exercise is also useful for training purposes. If those involved in sweat collection are unfamiliar with standard laboratory practises, for example nurses, training should be supervised by laboratory scientists and certified by senior members of the laboratory staff responsible for sweat testing. Since frequency of test performance is expected to contribute to competence, we support the CLSI guideline's recommendation of collectors performing at least 10 sweat tests each year.

Details regarding training must be fully documented and training records kept up-to-date in accordance with local audit agency requirements. It is desirable that the regular accreditation of laboratories by such agencies include an assessor with expertise in sweat testing.

False-negative and false-positive results can occur from any of the following:

- patient nutrition and hydration status
- skin contamination or failure to dry skin adequately
- inadequate sweat collection or sweat secretory rate
- evaporation during collection, transfer and transport
- improper method selection and performance
- poor technical competency

**Recommendation 17.** Each person trained to carry out sweat testing should perform at least 10 sweat tests annually, and detailed training records must be fully documented.

**Recommendation 18.** Training records should be kept up-to-date in accordance with local audit agency requirements.

**Recommendation 19.** Local accreditation authorities should ensure assessors with expertise in sweat testing are included in the assessor panel.

### IQC and EQA of the Analytical Process

Electrolyte solutions of known sodium and/or potassium chloride concentration should be used as IQC. When using gauze pads or filter paper, the IQC material should be added to the paper or gauze and then analysed with the patient samples. For the Wescor system, it is acceptable to analyse the IQC material directly. It is recommended that two levels of IQC be analysed with each batch of patient samples. It is

also recommended that one of these controls is close to the decision level for chloride concentration (i.e. 40 mmol/L) and the other is in the abnormal range.

Between-batch coefficient of variation (CV) for chloride measurement should be 5% or less at a concentration of 40–50 mmol/L.

### **Sweat Transport from Remote Laboratories**

One response to the RCPAQAP audits has been the increasing number of laboratories collecting sweat locally but sending it to central laboratories for analysis. This avoids patients needing to be sent long distances for testing. However it must be remembered that sweat stimulation and collection is the component of the test requiring the most standardisation. There is limited research on the maintenance of sample integrity during transport.<sup>44,45</sup> Current studies indicate that the traditional Gibson and Cooke sweat collection is only stable during transport for up to 6 h, whilst the sweat collected using the Macroduct® system is stable for up to 48 h; being lower than the storage guidelines provided elsewhere.<sup>45</sup> It is recommended that laboratories engage in this practise only if it has been experimentally demonstrated that the samples remain stable during transport.

**Recommendation 20.** Local collection of sweat with transport to another laboratory for analysis requires experimental demonstration that the samples remain stable during transport.

### **External Quality Assurance**

Laboratories undertaking sweat testing in Australasia must participate in a suitable EQA scheme. EQA can assess only the analytical component of sweat testing, and not the stimulation and collection components. Therefore errors arising from poor stimulation and/or collection practises are hard to identify. EQA can identify weighing errors, poorly performing methods, discrepancies in standardisation, calculation errors and errors in interpretation.

The RCPAQAP Chemical Pathology group have a quality assurance programme for sweat electrolytes. There are two cycles of testing per year consisting of six linearly-related samples distributed in duplicate. Currently the samples are aqueous salt solutions of NaCl and KCl. The range of chloride concentrations is 10–120 mmol/L. Part of the programme also looks at the interpretation of the results. This is the qualitative aspect of the report and currently there is a choice of ‘negative’, ‘equivocal’ or ‘positive’. Using these data, a cumulative summary report is produced graphically, representing the number of results returned and the interpretation selected by the laboratories.

### **Biological Variation**

There is significant variation between sweat tests performed on the same individual, whether performed at the same time on two sites, or on different occasions.<sup>28,46–49</sup> Quite apart from other causes of variation between tests, a major part of this has been shown to be biological variation within an individual, the causes of which have not been defined but may include physiological differences in adjacent skin sites and state of hydration. The total CV of repeat tests is at least 20%.<sup>28</sup> For the majority of patients, the chloride is either very low or, in a few cases, very high and variations of this magnitude will not affect the interpretation significantly. However for those with intermediate sweat chloride values, these variations could lead to misclassification. Patients with intermediate sweat test results should be referred to a clinician with experience in the diagnosis of CF.

### **Conclusions**

The CLSI and UK guidelines provide a good foundation for standardisation of the sweat test. Attention to each step is required to minimise variations, in particular the steps of sweat collection that are not assessed in the RCPAQAP. We encourage all laboratories in Australia and NZ performing sweat tests to familiarise themselves with these guidelines and our recommendations. Furthermore, we encourage all laboratories performing sweat tests in Australia and NZ to participate in the RCPAQAP. There is on-going research on many details of the sweat test and regular evaluation of the decision limits for the diagnosis of CF which will support the evidence base for our recommendations.

**Acknowledgements:** A draft of the 20 recommendations in this revised guideline was presented by RG and JM for discussion at the Ellitech Group Melbourne User Group Meeting, conducted on 11 September 2017 at the Pullman Hotel in Albert Park, Vic., Australia. We wish to thank all the meeting participants who represented many sweat testing laboratories in Australia for their input and support for this revised guideline.

**Competing Interests:** None declared.



**Table.** Comparison of guidelines for sweat testing. The design of this table was adapted.<sup>50</sup>

	Australia 1st Edition, 2006	CLSI 3rd Edition, 2009	United Kingdom 2nd Edition, 2014	CF Foundation 2nd Edition, 2017	Australasia 2nd Edition, 2017
<b>Pre-pre-analytical</b>					
Minimum age/weight of patient	>2 wk of age  AND > 3 kg	≥2 wk of age  AND > 2 kg OR 48 h of age if clinically important and sufficient sweat collected	≥2 wk of age  AND > 2 kg OR 7 d of age if clinically important and sufficient sweat collected	≥36 wk of corrected gestational age AND >2 kg	Recommendation 7
<b>Pre-analytical</b>					
Medium of collection	Washed filter paper or gauze  OR Wescor Macroduct® collectors.	Gauze or filter paper low in sodium and chloride  OR Microbore tubing.	Chloride free filter paper or gauze  OR Wescor Macroduct® disposable collectors	Sweat chloride testing should be performed according to approved procedural guidelines published in established, international protocols such as the CLSI 2009 Guideline.	Recommendation 10

	Australia 1st Edition, 2006	CLSI 3rd Edition, 2009	United Kingdom 2nd Edition, 2014	CF Foundation 2nd Edition, 2017	Australasia 2nd Edition, 2017
Site of collection	The flexor surface of either forearm is preferred site for sweat collection. Other sites e.g. upper arm, thigh or calf, may rarely be used if the arms are too small.	Sweat can be collected, with equivalent electrolyte concentration, from the lower arm or thigh; although the density of sweat glands is less in the thigh, making it a less optimal collection site. If leg is used, positive electrode should be placed in the inner thigh and the negative electrode.			
Current	4 mA	2.5–4 mA	1.5–4 mA	Sweat chloride testing should be performed according to approved procedural guidelines published in established, international protocols such as the CLSI 2009 Guideline.	
Stimulation time	5 min	5 min	3–5 min		
Collection time	20 – 30 minutes	Max 30 minutes	20–30 min (unless Macroduct® tubing is full earlier)		
Minimum weight/volume	≥1 g/m <sup>2</sup> /min 0.075 g – Gibson Cook	Exceed 1 g/m <sup>2</sup> /min 0.075 g - Gauze/Filter paper	≥1 g/m <sup>2</sup> /min		
Storage	15 µL – Microbore tubing No information available within guideline	15 µL – Microbore tubing Stable for 72 h.	Stable for 72 h at 4°C on Gauze pads.	Sweat chloride analysis should be performed within a few hours of sweat collection.	Not one of the 20 recommendations – refer to CLSI and UK guidelines.

Transport	Gauze or filter paper in a vial.	Macroduct® collection, either in Macroduct® tubing or capped PCR tubes for 72 h at 4°C.	No information available within guideline.	Sweat may be collected at remote sites and transported to the laboratory for analysis provided there is attention to storage details.	No information available within guideline.	Recommendation 20
	Microcentrifuge tubes	If liquid is stored at higher temperatures, PCR tubes must be used (max. 72 h).				
	Sample must not be stored in Microbore tubing.					
<b>Analytical</b>						
Screening method	Sweat conductivity	Sweat Conductivity (Outside of Cystic Fibrosis accredited centres only)	No information available within guideline.			Recommendation 6
Chloride method of analysis	Colorimetry, Coulometry and ISE	Titration with chloride meter. Caution using ISE. Other methods acceptable if validated.		Sweat conductivity using the Wescor Sweat-Chek equipment for > 6 months of age		Recommendation 11
	Flame photometry, ISE and atomic absorption.			Colorimetry, Coulometry and ISE		
Sodium (acceptable/unacceptable)	Osmolality	Direct reading in situ tests using ISEs or older electrical conductivity measurements, or measurements of osmolality or sodium are not acceptable as diagnostic tests.		Measurement not recommended.		Recommendation 5
Unacceptable analytes				sodium / potassium / osmolality / Wescor Nanoduct		

	Australia 1st Edition, 2006	CLSI 3rd Edition, 2009	United Kingdom 2nd Edition, 2014	CF Foundation 2nd Edition, 2017	Australasia 2nd Edition, 2017
<b>Post-analytical</b>					
Sweat chloride reference intervals	<40 mmol/L CF unlikely but unclear 40–60 mmol/L equivocal	Infants: ≥60 mmol/L indicative of CF. 30–59 mmol/L intermediate. ≤29 mmol/L normal.	<6 months 30–60 mmol/L equivocal;	In individuals with positive newborn screen, sweat chloride <30 mmol/L indicates CF is unlikely. 82% individuals with clinical features that may be consistent with CF who have sweat chloride <30 mmol/L indicates that CF is less likely.	Recommendations 13 & 14
	<40 mmol/L CF unlikely but unclear. 40–60 mmol/L equivocal.	Non-Infant: ≥60 mmol/L indicative of CF. 40–59 mmol/L intermediate. ≤39 mmol/L normal.	> 6months: 40–60 mmol/L	Individuals presenting with positive newborn screen, symptoms of CF, or positive family history, and sweat chloride values in intermediate range (30–59 mmol/L) on 2 separate occasions may have CF. They should be considered for extended CFTR gene analysis and/or CFTR functional analysis.	
	>60 mmol/L supports CF diagnosis	≥60 mmol/L indicative of CF.	>60 mmol/L supports CF diagnosis.	In individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history, a diagnosis of CF can be made if sweat chloride value is ≥60 mmol/L.	



Recommendation 15

Sweat conductivity  
reference intervals

Conductivity 50–80 mmol/L may be due to CF; all patients with conductivities >50 mmol/L should have measurement of sweat chloride concentration. Conductivity >80 mmol/L very likely to be due to CF.

Sweat conductivity value  $\geq 50$  mmol/L (equivalent NaCl) should have quantitative measurement of sweat chloride.

<50mmol/L (NaCl equivalents) unlikely to be CF

>90 mmol/L (NaCl equivalents) supports a diagnosis of CF.

Post-post-analytical

Sweat conductivity –  
screening & reflexing

N/A

Sweat conductivity may be performed at alternative sites. Sweat conductivity value  $\geq 50$  mmol/L (equivalent NaCl) should have quantitative measurement of sweat chloride.

In infants <6 m, sweat chloride must be measured even if conductivity levels are normal.

In infants >6 m, chloride concentration must be measured if borderline or positive conductivity levels are obtained.

Recommendation 15

	Australia 1st Edition, 2006	CLSI 3rd Edition, 2009	United Kingdom 2nd Edition, 2014	CF Foundation 2nd Edition, 2017	Australasia 2nd Edition, 2017
Sweat chloride follow-up	Patients with equivocal sweat chloride result should be referred to a physician specialising in the diagnosis and care of patients with CF. Diagnosis may be clarified by repeat sweat testing, genetic mutational analysis, measurement of nasal potential difference and in some instances, by tests of exocrine pancreatic function.			Results and interpretations should be reported to clinicians and parents or patients as soon as possible and certainly on the same day. Individuals who are screen-positive and meet sweat chloride criteria for CF diagnosis should undergo CFTR genetic testing if the CFTR genotype was not available through the screening process or is incomplete.	Recommendation 3

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